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Sexual Transmission of GB Virus C/Hepatitis G Virus

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Although it is established that infection with GB virus C (GBV-C) or hepatitis G virus (HGV) can be transmitted parenterally, the prevalence of GBV-C/HGV viremia in the general population (2–5%) is relatively high compared with other parenterally borne viruses such as hepatitis C virus. To investigate the possibility of sexual transmission of GBV-C/HGV, we determined the frequency of viremia by the polymerase chain reaction and serological reactivity to the E2 protein by ELISA in samples collected from individuals at risk for sexually transmitted diseases attending a city genitourinary medicine clinic. GBV-C/HGV viremia was detected in 27 of 87 male homosexuals (31%) and 9 of 50 prostitutes (18%), frequencies significantly greater than those in matched controls (2/63) and local blood donors (2.3%). Among nonviremic individuals, a high frequency of serological reactivity to the E2 protein of GBV-C/HGV was also observed in the risk groups (male homosexuals: 14/60; prostitutes: 11/41), although these figures are likely to be underestimates of the frequency of past infection as detectable anti-E2 reactivity may attenuate rapidly over time following resolution of infection. Infection with GBV-C/HGV was more frequent among those coinfected with human immunodeficiency virus type 1. Among male homosexuals from whom retrospective samples were available, evidence for de novo infection was found in 9 of 22 individuals over a mean sampling time of 2.9 years, predicting an annualized incidence of GBV-C/HGV infection of approximately 11% in this group. The high prevalence and incidence of GBV-C/HGV infection in these individuals and prostitutes provides strong evidence for its spread by sexual contact. Further studies are required to investigate the mechanism of its transmission and the clinical significance of acute and persistent infection in these risk groups. ***J. Med. Virol. 55:203–208, 1998.***

KEY WORDS: hepatitis C virus; homosexual; prostitute; HGV; GBV-C; anti-E2 ELISA

INTRODUCTION

GB virus C (GBV-C) or hepatitis G virus (HGV) is a recently discovered member of the flavivirus family [Simons et al., 1995; Leary et al., 1996; Linnen et al., 1996]. Its genome comprises approximately 9,400 nucleotides of positive-stranded RNA, with a genome organization similar to that of hepatitis C virus (HCV) and other members of the *Flaviviridae.* GBV-C/HGV infects humans, a proportion of whom become persistently viremic.

The role of GBV-C/HGV in the etiology of liver has been investigated intensively since its discovery. There is little evidence for clinically or biochemically apparent liver disease in those infected by blood transfusion [Wang et al., 1996; Alter et al., 1997a, 1997b; Yashina et al., 1997], and despite initial reports to the contrary [Yoshiba et al., 1995; Tameda et al., 1996], there is little evidence for an etiological role in fulminant hepatitis [Kao et al., 1996; Michitaka et al., 1996; Haydon et al., 1997; Kanda et al., 1997]. Similar difficulties surround the interpretation of studies investigating the role of GBV-C/HGV in chronic persistent hepatitis of unknown etiology. Although early reports indicated a significantly higher frequency of GBV-C/HGV infection in patients with non-A/non-E hepatitis [Colombatto et al., 1996; Fiordalisi et al., 1996; Linnen et al., 1996], later studies have largely discounted this association [Moaven et al., 1996a, 1996b; Abe et al., 1997; Alter et al., 1997a, 1997b; Feucht et al., 1997; Ross et al., 1997; Sarrazin et al., 1997; Sugai et al., 1997; Tanaka et al., 1997; Thomas et al., 1997; Zhang et al., 1997]. A difficulty in understanding the possible pathogenic significance of GBV-C/HGV arises from current ignorance of

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the target cell types infected with GBV-C/HGV in vivo; in contrast to an early report [Saito et al., 1997], current evidence suggests that this virus is not hepatotropic [Kudo et al., 1997; Laskus et al., 1997; Mellor et al., 1998], and therefore a wider degree of possible disease associations involving other organ systems should be considered.

While both GBV-C/HGV and HCV can be transmitted parenterally [Kim et al., 1995; Aikawa et al., 1996; Linnen et al., 1996; Nubling et al., 1996], infection with GBV-C/HGV is far more prevalent in the general population [Alter, 1996]. For example, among Edinburgh blood donors, less than 0.1% currently show evidence of HCV infection, while between 2–3% are viremic for GBV-C/HGV [Jarvis et al., 1996; Blair et al., unpublished results], frequencies comparable to other countries [Linnen et al., 1996; Moaven et al., 1996; Wang et al., 1996]. Maintenance of virus at this level in the general population is likely to require an effective nonparenteral route of transmission.

Reports of a higher frequency of GBV-C/HGV infection in prostitutes and in male homosexuals suggest that GBV-C/HGV can be sexually transmitted [Stark et al., 1996; Kao et al., 1997; Wu et al., 1997]. To investigate this hypothesis further, the prevalence of serumborne GBV-C/HGV RNA was examined among prostitutes and homosexual men in Edinburgh. These individuals were also tested for antibody to the E2 protein of GBVC/HGV by ELISA, which, combined with polymerase chain reaction (PCR) testing, allows an estimate of the total exposure to GBV-C/HGV. The frequencies of GBV-C/HGV infection were compared with those of other sexually and nonsexually transmitted virus infections, including hepatitis B virus (HBV), hepatitis C virus (HCV), human cytomegalovirus (CMV), and human immunodeficiency virus type 1 (HIV-1).

METHODS

Study Subjects

These comprised female prostitutes (n = 50, mean age 27), HIV-negative homosexual men (n = 52, mean age 26), and a separately selected group of HIV-positive male homosexuals (n = 35, mean age 29) attending the Genitourinary Medicine (GUM) clinic at the Royal Infirmary of Edinburgh. Prostitutes included in the study group were those who attended the GUM clinic for routine screening for sexually transmitted diseases [Scott et al., 1995]. All of the study subjects denied injecting drugs. A control group was selected from individuals requesting same-day HIV tests through the GUM clinic whose HIV test result was negative and who could be classed as low risk for sexual transmission (heterosexual, nondrug users, and no history of sexually transmitted diseases; n = 63, mean age 24).

Detection of GBV-C/HGV RNA

All samples for PCR were stored at –20°C before testing by PCR, and had not undergone multiple freeze–thaw cycles. RNA was prepared from 100 μl of serum using proteinase K/sodium dodecyl sulfate lysis buffer, followed by phenol/chloroform extraction, as described previously [Jarvis et al., 1994]. Reverse transcription was performed using one-fifth of this RNA. Weak positives (or ambiguous results) were confirmed by ultracentrifugation of 500 μl of serum (100,000 × g at 4°C for 1.5 hr) prior to extraction and using one-fifth of this nucleic acid preparation in the reverse transcription reaction. GBV-C/HGV-specific sequences were amplified by nested PCR using primers derived from the 5′NCR of GBV-C/HGV as detailed previously [Jarvis et al., 1996].

Antibody Detection

IgG antibodies to GBV-C/HGV E2-antigen were detected using the PLATE Anti-HGenv assay (Boehringer-Mannheim, Germany) [Dille et al., 1997]. Each sample was also tested for antibodies to hepatitis B virus core protein (Amerlite anti-HBc assay), human cytomegalovirus (Captia CMV-enzyme immunoassay, Centocor, London, UK), and for anti-HCV (VK48 anti-HCV immunoassay, Murex Biotech, Dartford, UK); samples seropositive for anti-HCV were confirmed using the Chiron recombinant immunoblot assay (RIBA, Chiron, Emeryville, CA).

RESULTS

Frequency of Past and Current GBV-C/HGV Infection

GBV-C/HGV RNA was detected in plasma samples from 2 of 63 individuals (3.2%) in the control group that comprised individuals negative for HIV-1 and at low risk for sexual transmission, similar to prevalences recorded among Edinburgh blood donors (3.2% (2/120) and 2.5% (23/1020) [Jarvis et al., 1996; Blair et al., unpublished data]. GBV-C/HGV viremia was found significantly more frequently among homosexual men (31% [27/87], $P < 0.0001$) and prostitutes (18% [9/50], $P < 0.01$) (Table I). Samples negative by PCR for GBV-C/HGV RNA were tested for anti-GBV-C/HGV antibody to estimate the extent of total exposure to GBV-C/HGV (RNA-positive + anti-E2-positive/PCR-negative). Evidence of past or current infection was found in 3/67 (4.8%) of the control group, and was significantly higher in prostitutes and homosexual men (40% [20/50], $P < 0.0001$, and 47% [41/87], $P < 0.0001$, respectively) (Table I).

The mean age of study subjects with any evidence of past or current infection with GBV-C/HGV was 26.5, compared with 25.5 for unexposed or uninfected individuals ($P = 0.038$). However, this association is likely to result from the higher mean age of the HIV-positive study subjects, since this group shows the highest frequency of total exposure to GBV-C/HGV (Table I). There were no significant differences in age between HGV-exposed and unexposed, HGV viremic or nonviremic, and HGV anti-E2-positive or -negative when each of the three risk groups was considered separately (all $P > 0.05$).

TABLE I. Frequency of GBV-C/HGV and Other Virus Infections in Study Subjects

Risk group	n	Mean age (range)	GBV-C/HGV viremia	anti-E2 antibody[a]	Total GBV-C/HGV exposure	CMV	HBV[b]	HCV
Controls	63	24 (15–44)	2/63 (3%)	1/61 (2%)	3/63 (5%)	15/63 (24%)	0/63 (0%)	0/63 (0%)
Prostitutes	50	27 (16–50)	9/50 (18%)	11/41 (27%)	20/50 (40%)	28/50 (56%)	3/38 (8%)	1/50 (2%)
Homosexuals, HIV-negative	52	26 (18–30)	9/52 (17%)	10/43 (23%)	19/52 (37%)	32/52 (62%)	7/25 (28%)	1/52 (2%)
Homosexuals, HIV-positive	35	29 (23–36)	18/35 (51%)	4/17 (24%)	22/35 (63%)	33/35 (94%)	12/25 (48%)	1/35 (3%)

[a]Frequency of reactivity to GBV-C/HGV E2 protein among samples negative by PCR.
[b]Frequency of anti-HBc reactivity in nonimmunized study subjects; all samples negative for HBsAg.

Comparison of GBV-C/HGV Prevalence With Other Virological Markers

Infection with GBV-C/HGV was more frequent among individuals infected with HIV-1 (Table I). Among homosexual men, the frequency of GBV-C/HGV viremia in HIV-positive individuals was 51% (18/35), compared with 17% (9/52) in those who were HIV-uninfected ($P < 0.001$). Combined with antibody results, there was also evidence for a higher frequency of total exposure in HIV-positive individuals (63% [22/35] compared with 37% [19/52] among HIV-negatives; $P < 0.02$). However, among homosexual men with evidence of past or current GBV-C/HGV infection, GBV-C/HGV viremia was detectable in a higher proportion of HIV-positive homosexual men (18 PCR+, 4 anti-E2+; n = 35) than HIV-negative homosexual men (9 PCR+, 10 anti-E2+; n = 52) or prostitutes (9 PCR+, 11 anti-E2+; n = 50). In addition, absolute levels of anti-E2 antibody in serum from HIV-positive individuals who had cleared GBV-C/HGV infection were lower than observed for HIV-negative individuals ($P = 0.05$). The mean optical density (OD) at 405 nm in the anti-E2 ELISA for HIV-positive homosexual men, who were positive for anti-GBV-C/HGV E2 antibody, was 0.602 (+/–0.131; range 0.514 to 0.795, n = 4), compared to 1.138 (+/–0.545; range 0.356 to 2.0, n = 10) for HIV-negative homosexual men, 1.028 (+/–0.523; range 0.357 to 1.839, n = 11) for prostitutes, and 1.084 (n = 1) for the control group.

The frequency of HCV infection was low in each study group (0–3%), consistent with a low frequency of past parenteral exposure. Among those not previously immunized against HBV, a higher frequency of past infection with HBV was found in HIV-positive homosexual men (48%), compared with HIV-negative homosexual men (28%), prostitutes (8%), or controls (0%). Because of the low parenteral exposure of each group, and the low frequency of vertically acquired HBV infections in the United Kingdom, the differing frequencies of past HBV infection are likely to reflect differences in the degree of sexual exposure between (nonimmunized) individuals in the different risk groups. There was a similar, but less marked, gradation in CMV prevalence among the different risk groups (HIV-positive gay men: 94%; HIV-negative gay men: 62%; prostitutes: 56%; and controls: 24%).

Look-Back Study

To investigate the incidence of GBV-C/HGV infection, we compared the frequency of GBV-C/HGV viremia and anti-E2 reactivity in available samples collected 3 years previous to the index sample from 35 of the homosexual men (17 HIV-positive, mean interval 3.2 years, range 2–6 years; 18 HIV-negative, mean interval 2.9 years, range 2–4 years; $P = 0.428$, not significant). Index samples from 5 of the HIV-positive and 8 of the HIV-negative homosexual men were PCR-negative, anti-E2-negative; 1 and 2 individuals were anti-E2-positive; and 11 and 8 individuals were PCR-positive, respectively.

Among the 13 PCR-negative, anti-E2-negative individuals, each previously collected sample was also negative in both assays. From the three individuals whose index samples were anti-E2-positive, one previous sample was anti-E2-positive and PCR-negative, one was anti-E2-negative and PCR-negative, and one was PCR-positive. Among individuals whose index samples were GBV-C/HGV PCR-positive, previous samples from 5 of the 8 HIV-negative individuals were PCR-negative and anti-E2-negative, while 3 were PCR-positive. A higher frequency of persistent infection was observed among HIV-positive individuals, where 7 of the 11 retrospective samples were PCR-positive. Overall, 9 cases out of 22 male homosexuals became PCR-positive over a mean sampling interval of 2.9 years (15% per year). Among individuals whose samples were used in the calculation of incidence, 9 of 22 were PCR-positive, similar to the proportion of PCR-positive individuals in the group as a whole (27/87; 31%). Adjusting for this difference in viremia, and making the assumption that the individuals from whom retrospective samples were available were representative of the group as a whole, the annualized incidence of GBV-C/HGV infection in the male homosexual risk group was 11% per year.

DISCUSSION

The high frequency and incidence of infection with GBV-C/HGV among individuals at high risk for sexually transmitted diseases is consistent with previous studies of GUM clinic attendees [Stark et al., 1996; Fiordalisi et al., 1997; Kao et al., 1997; Wu et al., 1997]. The specific association of GBV-C/HGV infection with

the degree of sexual exposure was inferred from the frequencies of past infection with HBV in the HIV-positive homosexual men (48%), HIV-negative homosexual men (28%), prostitutes (8%), and controls (0%). Parenteral exposure was an unlikely alternative source of infection because such risk factors were not elicited by patient interview and the frequency of infection with HCV was low in GBVC/HGV-exposed individuals.

No association was found between infection with GBV-C/HGV and age [Fiordalisi et al., 1997]. This may result from a relative insensitivity of the E2 ELISA (see below) and the lack of a close association between age and the degree of sexual exposure. In studies where exposure has been quantified, an association with the frequency of infection has been reported. For example, homosexual men with 100 previous sexual partners showed a significantly lower frequency of infection (8%) than those with >100 partners (21%) [Fiordalisi et al., 1997]. Similarly, the mean duration of sexual exposure of PCR-positive prostitutes in Taiwan was 6.5 years, significantly greater than 4.5 years among those who were PCR-negative [Kao et al., 1997]. However, a second study from Taiwan showed a lower mean age (28) of PCR-positive prostitutes than PCR-negatives [Wu et al., 1997]. Given the frequency with which GBV-C/HGV infection may spontaneously resolve, a better indication of the relation between sexual exposure and infection is likely to be obtained by serological testing in combination with PCR. Indeed, clearance of GBV-C/HGV may explain the low frequency of infection detected by PCR among partners of viremic intravenous drug users (IVDUs) [Halfon et al., 1997]. The finding that only 1 of 7 partners of IVDUs was PCR-positive had been interpreted as indicating that GBV-C/HGV is inefficiently transmitted by sexual contact [Halfon et al., 1997]. However, in the absence of serological testing, these findings do not rule out past, resolved infection in all or a large proportion of those who were PCR-negative.

In the current study, combined frequencies of viremia and anti-E2 antibody were used to estimate total exposure to GBV-C/HGV. The ability of the E2 ELISA to detect all cases of past infection is unclear, particularly as the ODs observed in some study subjects, especially those immunosuppressed through concurrent HIV-1 infection, were low and in many cases close to the cutoff of the assay. In a longitudinal study of hemophiliacs exposed to GBV-C/HGV through the use of nonvirally inactivated clotting factor concentrates, we have found that among 30 individuals with antibody to E2 by the Boehringer assay, 17 (57%) became seronegative after 10 years of follow-up [Jarvis et al., unpublished data]. A separate, indirect indication of the insensitivity of the Boehringer anti-E2 ELISA is provided by the low ratio of antibody reactivity to PCR positivity. Among the 201 study subjects, comparable numbers were found of anti-E2 reactive samples and PCR-positive samples (26:38, or 22:20 excluding HIV-positive individuals). This is lower than the ratios of anti-E2 reactivity to PCR-positivity of 2:1 among blood donors and 5:1 among IVDUs using a new ELISA based on E2 glycoprotein expressed in mammalian cells [Gutierrez et al., 1997]. These observations suggest that the combination of PCR and Boehringer anti-E2 ELISA results may underestimate the true frequency of exposure to GBV-C/HGV in each of the risk groups. This is particularly likely among the immunosuppressed HIV-infected homosexual men, given the high frequency of viremia (51%) relative to serological reactivity (24%), and the lower OD readings in the ELISA of the positive specimens. It is therefore possible that past or current infection with GBV-C/HGV may be close to universal in this group.

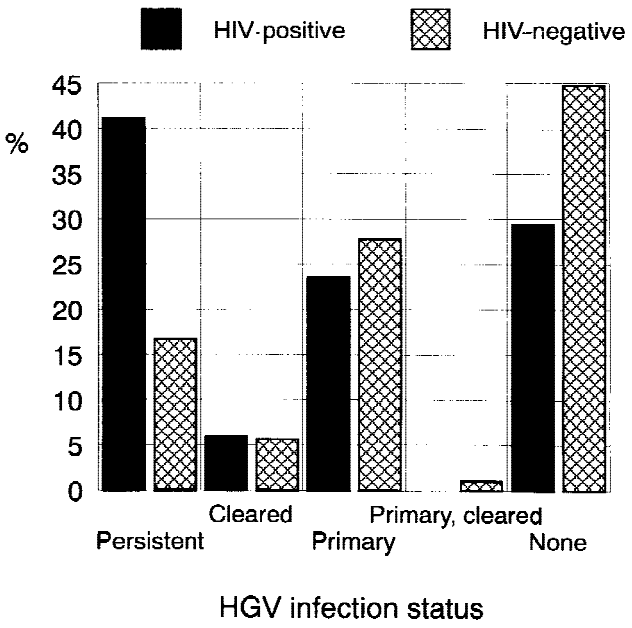

Fig. 1. Comparison of HGV status between HIV-positive (n = 17) and HIV-negative (n = 18) study subjects over look-back period of 3–4 years. Persistent denotes GBV-C/HGV PCR-positive for duration of study period; cleared: PCR-positive at start of study period, became PCR-negative on follow-up; primary: became PCR-positive during study period; primary, cleared: became PCR-positive during study period, followed by clearance (one case); and none: PCR-negative throughout study period.

An additional or alternative explanation for the high frequency of viremia among HIV-positive individuals is that the immunosuppression that accompanies HIV-1 infection may impair the ability of the immune system to clear infection with GBV-C/HGV. This hypothesis is supported by the finding of a higher frequency of persistent GBV-C/HGV viremia among HIV-positive study subjects (Fig. 1). These findings are consistent with the higher frequencies reported previously of active infection with GBV-C/HGV among multiply transfused patients immunosuppressed through hematological malignant disease or renal dialysis [Kudo et al., 1996; Neilson et al., 1996].

Despite the evidence for increased frequencies of GBV-C/HGV infection in association with sexual exposure, the mechanism of transmission remains unclear. Transmission may require unprotected sexual intercourse, or may simply result from intimate contact. The

finding that the frequency of GBV-C/HGV infection increased in proportion to past HBV infection (Table I) suggests a requirement for sexual contact to transmit infection, although barrier contraception is widely used among prostitutes in Edinburgh [Scott et al., 1995]. More definitive evidence for the mechanism of transmission will require, however, more detailed longitudinal studies of discordant couples, and a case-by-case analysis of the timing of transmission with specific types of exposure.

In summary, sexual exposure is likely to be a route of GBV-C/HGV transmission, and would explain the relatively high frequency of active and past infection in blood donors without evidence for past parenteral exposure. What remains undefined is the relative importance of this route of transmission and mother-to-child transmission [Feucht et al., 1996; Moaven et al., 1996b; Fischler et al., 1997]. By analogy with HBV, infection around the time of birth may facilitate persistent infection, and these long-term carriers may act as important reservoirs for the virus in the community. Such individuals may act as the ultimate source for infections acquired by sexual contact.

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